ON THE ECOLOGY OF THE LOWER MARINE FUNGI [1, 2]

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The lower marine fungi (*i.e.*, Myxomycetes and aquatic Phycomycetes) have generally been described as occurring on plant and animal hosts. While several forms have been described as saprophytes, the only genus known to occur on debris is *Labyrinthula* (Sparrow, 1936). All other described species are endobiotic or epibiotic with rhizoids penetrating living or dead host cells. They are also sporadic in occurrence. The application of a semi-quantitative plating technique to sea water has now established that lower fungi are far more common in littoral waters than previous studies indicate, and has suggested a new ecological niche for these fungi.

The plating technique which we used consisted of spreading samples of sea water with a bent glass rod on the surface of a solid isolation medium (Table I).

TABLE I

Isolation Medium

Sea water	80 ml./100 ml.
Gelatin hydrolysate	0.1%
Glucose (added aseptically)	0.1%
Liver 1:20	0.001%
B-vitamins	
Agar	1.5%
	adjusted to pH 7.5

Marine mineral base (Vishniac, 1955) was sometimes substituted for sea water. Gelatin hydrolysate and the B-vitamin mixture were prepared as by Vishniac and Watson (1953). Liver extract concentrate 1:20 was obtained from the Nutritional Biochemicals Co.

The moisture content of the medium is critical: the agar plates should be dried overnight, but not allowed to stand for more than two days. Just before use the plates are spread with 2000 units of Penicillin G (Squibb, buffered) and 0.5 mg. of dihydrostreptomycin sulfate (Wyeth) in concentrated aqueous solution. These plates will then absorb a 0.2-ml. sea water sample in an hour or two. After inoculation, the plates were incubated at 20 degrees or less. Such plates support the growth of lower fungi, yeasts, molds, and some diatoms, but few or no bacteria. We have found unfortunately incomplete suppression of bacterial growth when water samples taken from City Point, New Haven, near the sewage disposal plant,

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were plated. Colonies of lower fungi visible to the naked eye appear in a week or ten days. Colonies were counted at 30 × and further examined at 100 ×.

The results of spreading triplicate 0.2-ml. samples of sea water taken in June, 1956 from waters in and around Woods Hole, Mass. are given in Table II. The number of species represented is probably a minimum figure, since only colonies which were markedly distinct in color, texture, or size of thallus were picked for further study. Yeasts, molds and diatoms were not counted regularly. Yeasts were rare. From 0–5 colonies of molds, mainly *Penicillium* spp. of uncertain provenance, were found on those plates for which molds were counted. The presence of molds was not correlated with the presence of lower fungi.

It is evident from the poor agreement between triplicate platings in Table II that this technique is quantitative only within an order of magnitude. The procedure suffers from the following defects:

TABLE II

Occurrence of lower fungi in sea water

Origin of sample	Colonies/plate	No. species
1. Sea water tap, W.H.O.I.	0, 0, 0	0
2. Great Harbor	0, 0, 0	0
3. Eel Pond	94, 34, 20	7
Tap water, from bowls with algae		
4. Algae washed ca. 5 hrs.	23, 8, 20	5
5. Algae washed ca. 24 hrs. (another collection)	15, 10, 1	6
Water expressed from algae		
6. Pilings, U. S. Fish and Wildlife Station	62, 5, 56	2
7. Rocks, Red Spindle (Grassy Island)	66, 214, 35	7

(1) Spreading the sample, necessitated by the aerobic nature and poor temperature tolerance of the desired forms, entails the loss of 3 to 8% of a 0.2-ml. sample. The amount of sample remaining on the glass rod was determined by weighing the salt remaining on the glass rod after spreading a 20% NaCl solution.

(2) Either a thallus or a spore may give rise to a colony. The ecologic implications of the presence of a thallus or a spore in the sample are quite different.

(3) A thallus may produce zoospores in the interval between spreading and drying of the sample, giving rise to several colonies. The occasional appearance of groups of colonies of the same form on a plate was presumed to have this cause.

(4) Not every viable spore or thallus present in the sample may give rise to a colony. The conditions provided for growth were chosen after study of a limited number of marine forms—members of the genera *Labyrinthula, Sirolpidium, Thraustochytrium,* and three unidentified isolates. Obviously, forms with other requirements may exist. We have been particularly interested in assessing the probable extent of the selective action of the medium.

It is probable that forms with additional nutritional requirements would grow sufficiently on the medium used to form a countable colony. The ability of an individual organism, previously well nourished, to form a colony in the absence of required nutrients is inversely proportional to the quantity of nutrient required. Generally, absence of a suitable carbon source is felt first, amino acids required as

growth factors next, and lastly the absence of added vitamins—some of which may be stored in quantities sufficient for many generations. It is highly improbable that suitable carbon sources for any of the fungi with which we were concerned were lacking. As a source of amino acids, gelatin hydrolysate is inferior, since it is poor in methionine and in the aromatic amino acids. Nevertheless, we have maintained methionine-requiring Phycomycetes on gelatin hydrolysate media for over a year of semi-monthly transfers. The feasibility of isolating forms requiring growth factors not present in this medium was demonstrated by the isolation of sterol-requiring *Labyrinthulas* from two of the sea water samples used in this study (4 and 7, Table II). Syntrophism may occur (though no obvious examples were seen) on these plates.

An experimental approach to this problem was made by plating a sample of sea water from *Ulva* colonies on pilings at City Point, New Haven, in triplicate on the isolation medium, on the isolation medium without liver extract, on the isolation medium without liver extract, vitamins, or glucose, and on a medium containing glucose, glutamate, and thiamine as its only organic constituents. At the same time isolates known to require liver extract and known to require glucose, amino acids, and vitamins were plated on these media. Of the media used, only the glucose-glutamate-thiamine agar gave significantly lower sea water counts. This medium also failed to support typical colony formation by isolates known to require growth factors. The isolates requiring liver extract failed to form typical colonies on the medium from which liver, glucose, and vitamins were omitted also, though the omission of liver extract alone did not affect their growth under the conditions used.

It is, on the other hand, quite possible that fungi exist which did not form recognizable colonies because they were inhibited by the ingredients of the medium. Representative colonies were picked from the isolation plates made at Woods Hole into tubes of semi-solid (0.1% agar) isolation medium for further study. It then developed that, in semi-solid media, each of the organic constituents of the medium was somewhat inhibitory to some of the fungi isolated. The results of the comparative plating of City Point sea water outlined above and of similarly plating isolates known to be inhibited by ingredients of the isolation medium indicated that, for these fungi, the inhibitions are relieved by growth on a solid agar surface. The validity of this conclusion must be restricted to the fungi examined.

In principle, these defects are not unique to our procedure; they require restatement here because this is the first application of plating techniques to the lower marine fungi, indeed to ecologic studies of any aquatic Phycomycetes, and because they bear on the conclusions to be drawn from our results. The results of Table II suggest, first, that the presence of these fungi is correlated with the organic content of the water examined, since the highly polluted Eel Pond is richer than Great Harbor (on the incoming tide). Second, in Great Harbor (and in the Hole), fungi are associated with algae: they may be isolated from water taken from finger bowls in which algae were being kept under a constant drip of previously fungus-free tap water. They may be isolated from water squeezed by hand from masses of attached algae growing on rocks and pilings.

The organic content of polluted waters, such as those of the Eel Pond, could reasonably be expected to support a population of free-living non-filamentous fungal saprophytes, just as of bacterial saprophytes. We have calculated, from cell counts of representative cultures, that the amount of soluble organic material required to produce a single thallus of the common holocarpic or monocentric marine Phycomy-

cetes is of the order of 1 mμ g. But the development of techniques for establishing directly the presence of a free-living fungus population would be very desirable. Two instances of association with organic debris were noted. One colony of a monocentric Phycomycete was found on a plate on a stray grain of pine pollen. One species (an undescribed myxoid form here referred to as isolate "S"), of which 1–22 colonies were found on every plate containing lower fungi, formed colonies which were as often as not centered on a microscopic bit of nondescript organic debris.

In view of the known endo- and epibiotic habit of marine fungi, the apparent association with algae required further investigation. The algae with which the sea water samples of Table II were associated were examined microscopically for the presence of fungal thalli. As might have been expected from the experience

TABLE III

Fungi associated with algal surfaces

	No. fragments	No. species fungi/fragment
1. Algae from rocks at Red Spindle (Grassy Island)		
Ectocarpus	1	2
Antithamnion	8	1–3
Polysiphonia	4	1–3
Ceramium	3	1–3
2. Algae from rocks of Pine Island		
Ectocarpus	4	1–2
Elachistea	5	1
Punctaria	2	2
Chorda filum	1	3
Callithamnion	18	1–3
Antithamnion	4	2
	1	0
Polysiphonia	3	0
Bryopsis	10	1–2
	4	0

of previous investigators (see Petersen, 1905; Sparrow, 1934, 1936; Kobayashi and Ookubo, 1953), recognizable thalli were rare. The one species which Sparrow (1936) found epidemic later in the summer—*Ectrogella perforans* in *Licmophora*—was conspicuously absent. The only form found during these examinations was *Petersenia lobata* (?) on *Polysiphonia urceolata* (*fide* W. R. Taylor) collected at the Red Spindle and allowed to rest for 12 days in a finger bowl under dripping sea water (as suggested by Sparrow). Five days later the infection had disappeared.

The results of plating small pieces of algae showed, in marked contrast, that the algal surface not contaminated by lower fungi was rare. Bits of algae, usually strands approximately one cm. long, were cut from the collected plants, drawn gently over the edge of a petri dish to remove water and placed on the surface of the usual antibiotic-isolation medium agar. No fungal thalli (possibly because of their small size) could be recognized at 100 × on the algal fragments at the time of plating. Neither fungal thalli nor rhizoids were seen within the cells of these algae at any time, though the specimens were examined at 430 × at the end of the incubation period. The association of lower fungi with fragments of algae collected at the Red Spindle (Grassy Island) in Great Harbor and at Pine Island

(off Nonamesset Island, in the Hole) is shown in Table III. Molds and yeasts were observed fairly frequently; specimens in this series which were obscured by such forms have been omitted from the tabulation.

Are these fungi on the algal surface or in the surface film of water surrounding the algal fragment? If the surface film of water is, at a guess, about 0.01 ml., one would expect to find of the order of 1–10 viable units of lower fungi in it. The results of Table III are not inconsistent with this estimate, but are unfortunately not quantitative, because discrete colonies are rarely formed around algal fragments. But we are inclined to consider, for two reasons, that the fungi found in association with algal fragments resemble many of the marine bacteria in being present, as thalli, on the surface of the alga. First, we have attempted (in a limited number of trials) to wash the fungi away with sterile sea water. The results, even with such algae as *Bryopsis plumosa* and *Polysiphonia urceolata* which were free of the forest of hairs and epiphytes found on most marine algae, were poor. No more fragments were free of fungi after washing than before, and essentially the same type of fungi was present. Secondly, although both holocarpic and eucarpic Phycomycetes were among the forms associated with algal surfaces, the predominant form was, as in sea water samples, the myxoid surface-loving "S." Every algal fragment listed in Table III as having associated fungi had "S" associated with it. The association of "S" with bits of organic debris has already been noted. "S" was also found to be associated with two of six copepods plated as part of a rather unproductive plankton haul from Great Harbor. Another surface-loving form, *Labyrinthula* sp., occurred less frequently on *Polysiphonia*, *Ceramium*, and *Ectocarpus*.

Conclusions and Summary

Our data therefore suggest that the lower marine fungi occupy essentially the same ecologic niche as the marine saprophytic bacteria. These fungi can be found in suitably polluted sea water in numbers of the order of 1–500,000 viable units/liter but less than 5000/liter in more open waters of Woods Hole. The fungus count increases, as has long been noted for bacteria (Gazert, 1906), in the presence of macroscopic algae. They also resemble marine bacteria in their association with surfaces. As a group, these fungi differ from marine bacteria in being strongly aerobic. One may justifiably wonder as to the basis of their success in competition with bacteria in this niche. Studies, now in progress, of the individual species of fungi concerned may clarify this question.

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